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Bescheinigung | Certificate | Attestation

Die angehefteten Unterlagen stimmen mit der ursprünglich eingereichten Fassung der auf dem nächsten Blatt bezeichneten europäischen Patentanmeldung überein.

The attached documents are exact copies of the European patent application described on the following page, as originally filed.

Les documents fixés à cette attestation sont conformes à la version initialement déposée de la demande de brevet européen spécifiée à la page suivante.

Patentanmeldung Nr. | Patent application No. | Demande de brevet n°

98202470.5

PRIORITY DOCUMENT

SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.c.

Arlette Fiedler

A. Fiedler

DEN HAAG, DEN
THE HAGUE, 13/09/99
LA HAYE, LE

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Blatt 2 der Bescheinigung
Sheet 2 of the certificate
Page 2 de l'attestation

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Applicant(s):
Demandeur(s):
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Titre de l'invention:
Novel peptides for use in immunotherapy of autoimmune diseases

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NOVEL PEPTIDES FOR USE IN IMMUNOTHERAPY OF AUTOIMMUNE DISEASES

The invention relates to novel peptides, their use in treatment of chronic destruction of articular cartilage in autoimmune diseases, pharmaceutical compositions comprising said peptide, and a diagnostic method for the detection of autoreactive T cells in a test sample.

The immune system is established on a principle of discrimination between foreign antigens (non-self antigens) and autoantigens (self antigens, derived from the individuals own body) achieved by a build-in tolerance against the autoantigens.

The immune system protects individuals against foreign antigens and responds to exposure to a foreign antigen by activating specific cells such as T- and B lymphocytes and producing soluble factors like interleukins, antibodies and complement factors. The antigen to which the immune system responds is degraded by the antigen presenting cells (APCs) and a fragment of the antigen is expressed on the cell surface associated with a major histocompatibility complex (MHC) class II glycoprotein. The MHC-glycoprotein-antigen-fragment complex is presented to a T cell which by virtue of its T cell receptor recognizes the antigen fragment conjointly with the MHC class II protein to which it is bound. The T cell becomes activated, i.e. proliferates and/or produces interleukins, resulting in the expansion of the activated lymphocytes directed to the antigen under attack (Grey et al., Sci. Am., 261:38-46, 1989).

Self antigens are also continuously processed and presented as antigen fragments by the MHC glycoproteins to T cells (Jardetsky et al., Nature 353:326-329, 1991). Self recognition thus is intrinsic to the immune system. Under normal circumstances the immune system is tolerant to self antigens and activation of the immune response by these self antigens is avoided.

disadvantages of nonspecific immunosuppression makes this a highly unfavourable therapy.

The antigen-specific, nontoxic immunosuppression therapy provides a very attractive alternative for the nonspecific immunosuppression. This antigen-specific therapy involves the treatment of patients with the target autoantigen or with synthetic T cell-reactive peptides derived from the autoantigen. These synthetic peptides correspond to T cell epitopes of the autoantigen and can be used to induce specific T cell tolerance both to themselves and to the autoantigen. Although it seems paradoxical to desensitize the immune system with the very same antigen responsible for activating the immune system, the controlled adminstration of the target (auto)antigen can be very effective in desensitization of the immune system. Desensitization or immunological tolerance of the immune system is based on the long-observed phenomenon that animals which have been fed or have inhaled an antigen or epitope are less capable of developing a systemic immune response towards said antigen or epitope when said antigen or epitope is introduced via a systemic route.

The human cartilage glycoprotein-39 (HC gp-39) was previously identified as a target autoantigen in rheumatoid arthritis (RA) (Verheijden et al., Arthitis Rheum. 40:1115-1125, 1997). The strategy followed for identification of relevant auto-epitopes within HC gp-39 was based on the assumption that the DR4 or DR1 molecules predispose to RA (Gao et al., Arthitis Rheum. 33:939-946, 1990; Nelson et al., Rheumatoid Arthritis, In Proceedings of the Eleventh International Histocompatibility Workshop and Conference. Vol 1, Tsuji et al Ed, Oxford University Press, 1991) at two levels, firstly, by shaping the T cell repertoire and secondly, by determinant selection. The shared epitope found among the RA-associated DR molecules might be involved in selection of similar sets of peptides for presentation to T cells (Gregerson et al., Arthitis Rheum. 30:1205-1213, 1987). Putative binding sequences within the primary structure of HC gp-39 were identified by use of a DR4 (B1*0401) peptide binding motif (Verheijden et al., Arthitis Rheum. 40:1115-1125, 1997). HC gp-39, a protein of 362 amino acids, excluding the signal sequence (Hakala et al., J. Biol. Chem. 268:25803-25810, 1993), contains six regions accommodating this motif. Four peptides thus selected were synthesized and tested for binding the RA-associated DR1 and DR4 (B1*0401 and 0404) variants. All motif-based peptides, spanning residues 103-116, 259-275, 263-275 and 326-338 of HC gp-39, were found to bind with high relative affinity to DRB1*0401 molecules. The recognition of these peptides by peripheral blood T cells from RA patients and healthy donors was subsequently examined. All motif-

This epitope therefore is useful for tolerization of autoreactive T-cells with reactivity to HC gp-39 (263-275), YKL-39 (266-278) or their mimicry epitopes in rheumatoid arthritis patients.

It is an object of the invention to provide peptides which are able to induce systemic immunological tolerance, more in particular specific T cell tolerance, preferably to the responsible cartilage antigen in patients suffering from T cell-mediated cartilage destruction. The peptides of the present invention are characterized in that they comprise one or more of the amino acid sequences FTLASAETT (SEQ ID NO: 1). More specifically, a peptide according to the invention comprises HSFTLASAETTVG (SEQ ID NO: 2).

Also within the scope of the invention are multimers of the peptides according to the invention such as for example a dimer or trimer of the peptides according to the invention. A multimer according to the invention can either be a homomer, consisting of a multitude of the same peptide, or a heteromer consisting of different peptides.

The characteristic amino acid sequences of the peptides according to the invention can be flanked by random amino acid sequences. Preferred are flanking sequences, that have a stabilizing effect on the peptides, thus increasing their biological availability.

Human Cartilage glycoprotein 39 is a target autoantigen in RA patients which activates specific T cells, thus causing or mediating the inflammatory process. HC gp-39 derived peptides were predominantly recognized by autoreactive T cells from RA patients but rarely by T cells from healthy donors, thus indicating that HC gp-39 is an autoantigen in RA. The arthritogenic nature of HC gp-39 was further substantiated in the Balb/c mouse. A single, subcutaneous injection of said protein in Balb/c mice was able to initiate arthritic signs in the animals. The course of the HC gp-39- induced disease was characterized by relapses occuring periodically in fore paws and/or hind paws and gradually developed from a mild arthritis into a more severe form. Also, a symmetrical distribution of afflicted joints was observed which is, together with the observation of recurrent relapses, reminiscent of disease progression in arthritis, especially RA.

It was surprisingly found that the YKL-39 266-278 peptide was effective as a tolerogen. It will be clear to those skilled in the art that the peptides may be extended at either side of the peptide or at both sides and still exert the same immunological function. The extended part may be an amino acid sequence similar to the natural sequence of the protein YKL-39.

The peptides according to the invention can also be used to modulate lymphocytes that are reactive to antigens other than said autoantigen but are present in the same tissue as the autoantigen i.e. proteins or parts thereof comprising the peptide according to SEQ ID NO:1 or SEQ ID NO:2. By the induction of antigen-specific T-cell tolerance, autoimmune disorders can be treated by bystander suppression. More in general, the cells to be modulated are hematopoietic cells. In general, in order to function as a tolerogen the peptide must fulfill at least two conditions i.e. it must possess an immune modulating capacity and it must be expressed locally usually as part of a larger protein.

Thus, the present invention provides a method to treat patients suffering from inflammatory autoimmune diseases, by administration of a pharmaceutical preparation comprising the peptide according to the invention. Such patients may suffer from diseases like Graves' diseases, juvenile arthritis, primary glomerulonephritis, osteoarthritis, Sjögren's syndrome, myasthenia gravis, rheumatoid arthritis, Addison's disease, primary biliary sclerosis, uveitis, systemic lupus erythematosis, inflammatory bowel disease, multiple sclerosis or diabetes. The peptides according to the present invention therefore can be used in the preparation of a pharmaceutical to induce tolerance in patients suffering from these diseases.

Treatment of autoimmune disorders with the peptides according to the invention makes use of the fact that bystander suppression is induced to unrelated but co-localized antigens. The regulatory cells secrete in an antigen specific fashion pleiotropic proteins such as cytokines which may downmodulate the immune response.

According to the invention, patients suffering from T-cell mediated destruction of the articular cartilage can be treated with a therapeutical composition comprising one or more peptides according to the invention and a pharmaceutical acceptable carrier. Administration of the pharmaceutical composition according to the invention will induce systemic immunological tolerance, in particular tolerance of the specific autoreactive T cells of these patients, to the autoantigenic proteins in the articular cartilage under attack and other self antigens which display the identified MHC Class II binding T cell epitopes characterized or mimicked by the amino acid sequences of one or more of the peptides according to the invention. The induced tolerance thus will lead to a reduction of the local inflammatory response in the articular cartilage under attack.

Suitable administration routes are e.g. intramuscular injections, subcutaneous injections, intravenous injections or intraperitoneal injections, oral administration and nasal administration such as sprays.

It is another object of the invention to provide a method for detecting autoreactive T cells involved in the destruction of articular cartilage and test kits to be used in said method. Thus, the peptides according to the invention are also very suitable for use in a diagnostic method to detect the presence of activated autoreactive T cells involved in the chronic inflammation and destruction of the articular cartilage.

The diagnostic method according to the invention comprises the following steps:

a) isolation of the peripheral blood mononuclear cells (PBMC) from a blood sample of an individual,

b) culture said PBMC under suitable conditions,

c) incubation of said PBMC culture in the presence of one or more peptides according to the invention, and

d) detection of a response of T cells, for example a proliferative response, indicating the presence of activated autoreactive T cells in the individual.

The detection of a proliferative response of T cells can be detected by, for example, the incorporation of ^{3}H-thymidine.

Also within the scope of the invention are test kits which comprise one or more peptides according to the invention. These test kits are suitable for use in a diagnostic method according to the invention.

The following examples are illustrative for the invention and should in no way be interpreted as limiting the scope of the invention.

Table 1. Alignment of the HC gp-39 (263-275) sequence with the corresponding region in YKL-39 and macrophage Chitotriosidase

HCgp-39 263-275	R	S	F	T	L	A	S	S	E	T	G	V	G
YKL-39 266-278	H	S	F	T	L	A	S	A	E	T	T	V	G
Chi (269-282)	R	S	F	T	L	A	S	S	S	D	T	R	V	G

Example 2 Binding of peptides to HLA-DRB1*0401

The peptides from example 1 were tested for binding the DRB1*0401-encoded molecules. HLA-DR4 (DRB1*0401) molecules were purified from the homozygous EBV-transformed human B lymphoblastoid cell lines Huly138IC2 and the competition peptide HLA-DR binding assay was performed basically as described by Verheijden et al., 1997. The affinity of a given peptide for binding DRB1*0401-encoded molecules was related to competition with a marker peptide. This relative binding affinity was defined as the peptide concentration at which the signal was reduced to 50% (IC50). The HA-F peptide is a positive control (Hemagglutinin 307-319; PKFVKQNTLKLAT; at position 309 Y is substituted by F; SEQ ID NO:5). The peptide is known to have a high affinity for DRB1*0401 molecules.

As expected, the Chi(269-282) peptide was found to bind with high affintiy to DRB1*0401 (see table 2). The YKL-39 (266-278) peptide, which does not accommodate the effective DRB1*0401 peptide binding motif, bound with very high affinity to DR4 (B1*0401).

cells per ml. Cells were incubated in medium alone or in the presence of 10 or 50 µg/ml peptide antigen (YKL-39 (266-278)). Cultures were incubated for 6 days at 37 °C in a humidified atmosphere of 5% CO2. Cells were then suspended and 100 or 150 µl volumes of medium was distributed in 4-fold in wells of a 96-well roundbottomed plate. Cells were then pulsed with 0.5 µCi (1.85×10^4 Bq) [3H]thymidine ([3H]TdR) and 18 hr later incorporated radioactivity was measured. Results as shown in table 3 are expressed as stimulation indices (SI) (antigen-specific counts/background counts)

From Table 3a it can be concluded that the YKL-39 (266-278) epitope is readily recognized in RA patients. Table 3b indicates that recognition of YKL-39 (266-278)) by PBMC coincides with recognition of HC gp-39 (263-275) and HC gp-39 and furthermore that recognition of YKL-39 (266-278) is generally more pronounced than recognition of HC gp-39 (263-275).

Table 3a. Recognition of the YKL-39 (266-278) epitope by PBMC from RA patients.

Donor		typing	SI 10 µg/ml	SI 50 µg/ml
242-0.2	NR	0404/15	3	<2
337-0.2	R	0401/02	19	58
338-0.1	NR	03/14	<2	<2
454-0	R	0401/	9	9
456-0	R	ND	15	4
457-0	NR	ND	<2	<2
458-0	R	ND	4	27
459-0	R	ND	<2	25
460-0	NR	ND	3	<2

SI = antigen-specific counts/background counts. SI ≥ 5 are regarded positive R = responder, NR = non-responder

Table 4. Experimental set-up tolerization experiment

Pretreatment	sensibilisation	challenge	tolerance
none	HC gp-39 (263-275)	HC gp-39 (263-275)	no
saline	HC gp-39 (263-275)	HC gp-39 (263-275)	no
HC gp-39 (263-275)	HC gp-39 (263-275)	HC gp-39 (263-275)	yes
YKL-39 (266-278)	HC gp-39 (263-275)	HC gp-39 (263-275)	yes

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Phe Thr Leu Ala Ser Ala Glu Thr Thr
1 5

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

His Ser Phe Thr Leu Ala Ser Ala Glu Thr Thr Val Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 13 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Arg Ser Phe Thr Leu Ala Ser Ser Glu Thr Gly Val Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 14 amino acids
(B) TYPE: amino acid

Claims

1. Peptide having an amino acid sequence of 9-55 amino acid residues comprising the amino acid sequence FTLASAETT (SEQ ID NO:1).
2. Peptide according to claim 1 comprising the amino acid sequence HSFTLASAETTVG (SEQ ID NO:2).
3. Peptide according to claim 1 or 2 having an amino acid sequence of up to 25 amino acid residues.
4. Peptide according to claim 1 or 2 having the amino acid sequence FTLASAETT (SEQ ID NO:1) or HSFTLASAETTVG (SEQ ID NO:2).
5. Peptides according to any of the claims 1-4 for use as a therapeutical substance.
6. Pharmaceutical composition comprising one or more of the peptides according to claims 1-4, and a pharmaceutical acceptable carrier.
7. Use of one or more of the peptides according to claims 1-4 for the manufacture of a pharmaceutical preparation for the induction of specific T-cell tolerance to an autoantigen in patients suffering from autoimmune disorders, more specifically arthritis.
8. Diagnostic composition comprising one or more of the peptides according to any of the claims 1-4 and a detection agent.

Figure 1A

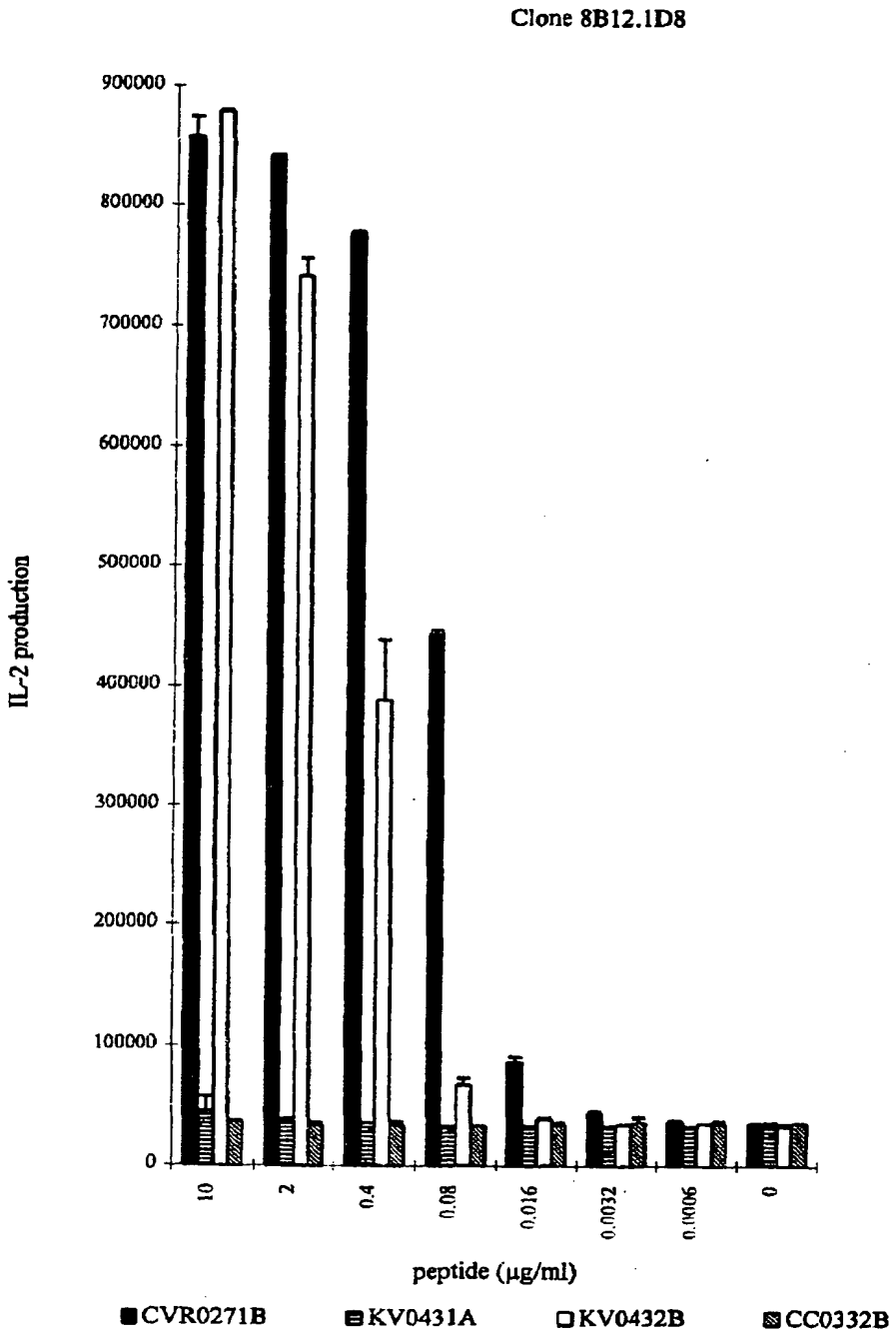
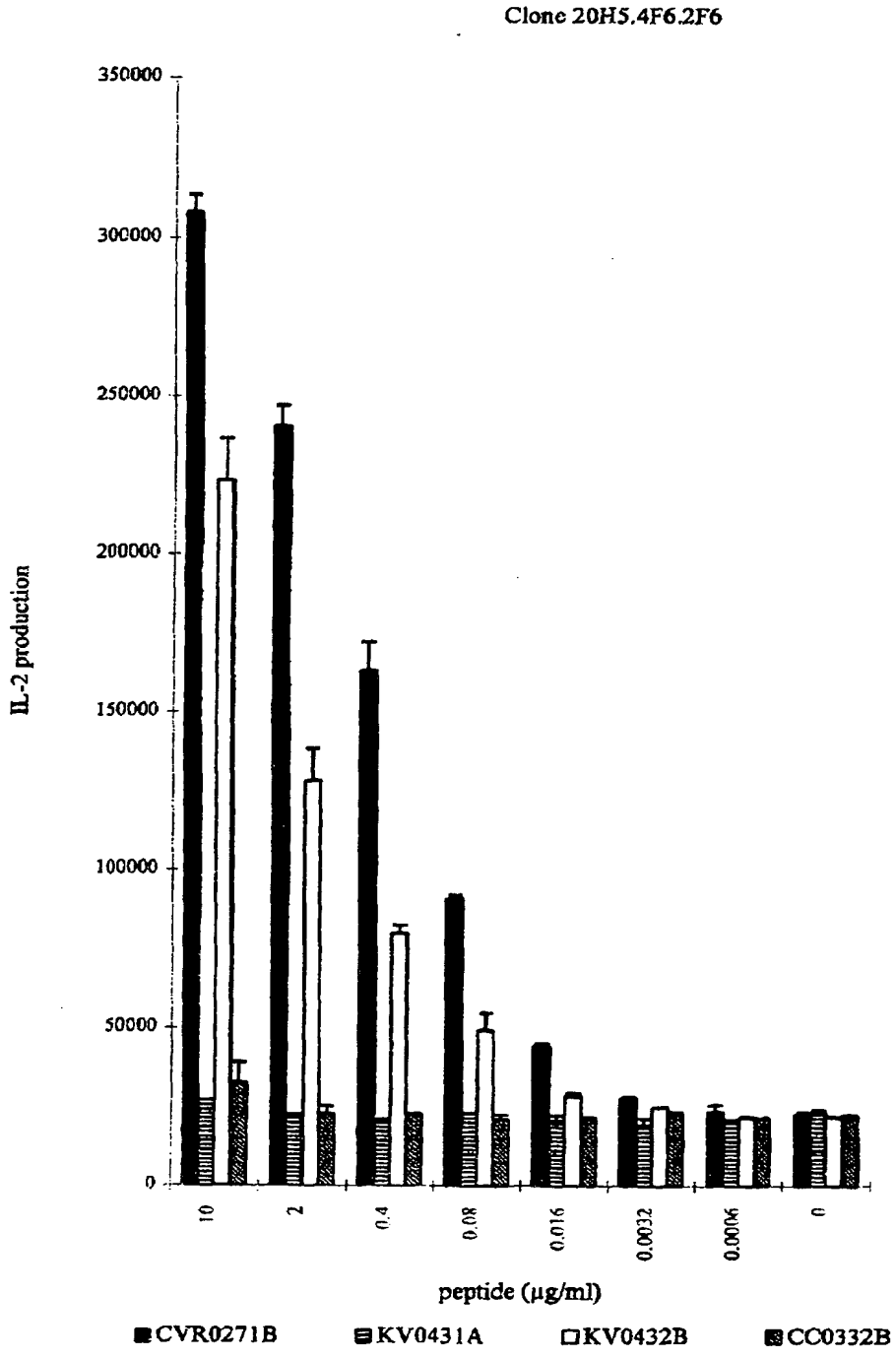


Figure 1C



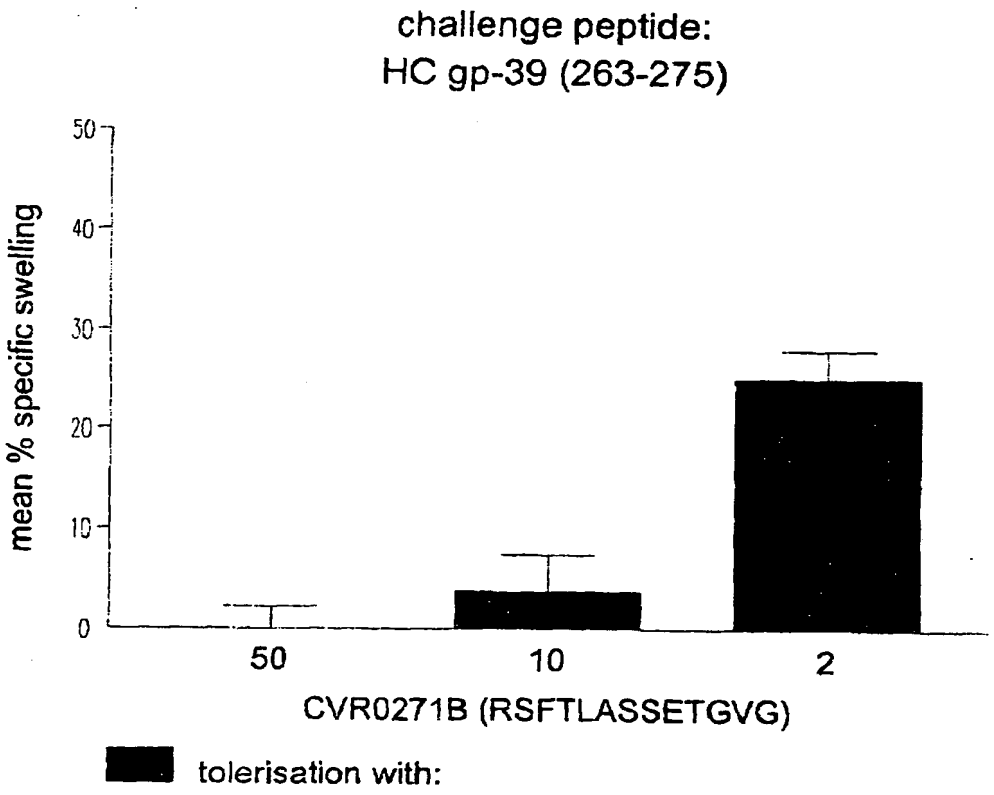


Figure 2, cont.

Abstract

The invention relates to the use of novel peptides in a peptide induced tolerance therapy to prevent autoimmunune disorders and in particular their use in treatment of chronic destruction of articular cartilage. The invention furthermore embraces pharmaceutical compositions comprising said peptides and a diagnostic method for the detection of autoreactive T cells in a test sample.

PCT/EP 99 /05051

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Patentanmeldung Nr. | Patent application No. | Demande de brevet n°

98305837.1

Der Präsident des Europäischen Patentamts;
Im Auftrag

For the President of the European Patent Office

Le Président de l'Office européen des brevets
p.o.

I.L.C. HATTEN-HECKMAN

DEN HAAG, DEN
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Blatt 2 d r Bescheinigung
Sheet 2 of the certificate
Page 2 de l'attestation

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Anmelder:
Applicant(s):
Demandeur(s):
Akzo Nobel N.V.
6824 BM Arnhem
NETHERLANDS

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alpha-Amino acid phenyl ester derivatives

In Anspruch genommene Priorität(en) / Priority(ies) claimed / Priorité(s) revendiquée(s)

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Remarks:
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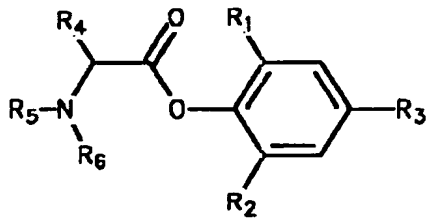
α-AMINO ACID PHENYL ESTER DERIVATIVES

The invention relates to α-amino acid phenyl ester derivatives, to pharmaceutical compositions containing the same, as well as to the use of these α-amino acid phenyl ester derivatives as hypnotics for the induction and maintenance of general anaesthesia.

It has been reported (G. Brancaccio and A. Larizza, Il Farmaco 1964, 19, 986-1002) that α-amino acid phenyl ester derivatives, wherein the amino group is either dialkylated or is part of an heterocyclic system (GB 1,102,011: Richardson-Merrell S.p.A.), possess local anaesthetic activity, with piperazinyl derivatives proving the most active. In GB 1,160,468 (May & Baker Ltd.) an α-amino acid phenyl ester wherein the amino group is part of a morpholinyl ring, i.e. 2,6-dimethoxyphenyl 2-morpholinopropionate, is disclosed as an intravenous general anaesthetic having a short duration of activity with rapid, smooth recovery. The hypnotic properties of this compound are attained at rather high dose levels and consequently there exists a need for new water soluble intravenous general anaesthetics with improved potency.

The present invention provides α-amino acid phenyl ester derivatives having the general formula I



Formula I

wherein

R_1 is (C_{1-3})alkyloxy;

R_2 is (C_{1-3})alkyl, (C_{1-3})alkyloxy or (C_{2-3})alkenyl;

R_3 is hydrogen, (C_{1-3})alkyl, (C_{1-3})alkyloxy or (C_{2-3})alkenyl;

R_4 is (C_{1-6})alkyl;

R_5 and R_6 ar independently (C_{1-6})alkyl, (C_{2-6})alkenyl, (C_{2-6})alkynyl or aralkyl, each of which may be optionally substituted with (C_{1-3})alkyloxy, (C_{1-3})alkyloxycarbonyl, cyano or NR_7R_8;

R_7 and R_8 are independently (C_{1-6})alkyl;

or a pharmaceutically acceptable salt thereof, with the exclusion of 2,6-dimethoxyph nyl 2-(diethylamino)propionate and 2,6-dimethoxyphenyl 2-(diethylamino)butyrate.

Since 2,6-dimethoxyphenyl 2-(diethylamino)propionate and 2,6-dimethoxyphenyl 2-(diethylamino)butyrate have been described as local anaesthetics by G. Brancaccio and A Larizza (vide supra), no protection is sought for these compounds per se.

The α-amino acid phenyl ester derivatives of formula I, having a dialkylated amino group, were surprisingly found to be potent intravenous hypnotics with quick onset, and a short duration of action with rapid, smooth recovery.

The term (C_{1-6})alkyl, as used in the definition of formula I, means a branched or unbranched alkyl goup having 1-6 carbon atoms, like hexyl, pentyl, isobutyl, tertiary butyl, propyl, isopropyl, ethyl and methyl.

The term (C_{1-3})alkyl means an alkyl group having 1-3 carbon atoms, like n-propyl, isopropyl, ethyl and methyl.

In the term (C_{1-3})alkyloxy as used in formula I, (C_{1-3})alkyl has the meaning as previously given, preferably methyl.

The term (C_{2-6})alkenyl means a branched or unbranched alkenyl group having 2-6 carbon atoms, like for example hexenyl, pentenyl, butenyl, 1,3-butadienyl, 1-methyl-propen-2-yl, propen-2-yl (allyl), propen-1-yl or ethenyl (vinyl). Alkenyl groups having at least 3 carbon atoms may be in the E- or Z-form, or a mixture thereof.

The term (C_{2-3})alkenyl means an alkenyl group having 2 or 3 carbon atoms, like propen-2-yl, propen-1-yl or ethenyl (vinyl).

The term (C_{2-6})alkynyl means a branched or unbranched alkynyl group having 2-6 carbon atoms, like hexynyl, pentynyl, butynyl, propyn-2-yl or ethynyl.

The term aralkyl means an aryl(C_{1-3})alkyl group, wherein alkyl means a bival nt carbon radical having 1-3 carbon atoms, such as methylene, ethan-1,2-diyl, propan-1,3-diyl,

ethyliden or propylidene, and wherein aryl means C_{6-12} aryl aromatic groups and includes one or two C_6-aromatic rings, like for example phenyl, naphthyl or biphenyl.

Preferred α-amino acid phenyl ester derivatives of the invention correspond to compounds having formula I wherein R_1 and R_2 are methoxy; and R_4 is (C_{2-3})alkyl, like ethyl, propyl or isopropyl, and wherein R_3, R_5 and R_6 have the previously given meanings.
More preferred are the compounds wherein R_1 and R_2 are methoxy, R_3 is hydrogen or methyl, R_4 is (C_{2-3})alkyl, and R_5 and R_6 are independently methoxyethyl or ethoxyethyl.
Especially preferred α-amino acid phenyl ester derivatives of the invention correspond to formula I wherein R_1 and R_2 are methoxy; R_3 is hydrogen or methyl; R_4 is ethyl; and R_5 and R_6 are methoxyethyl.

The compounds of formula I and their salts contain at least one centre of chirality, i.e. at the α-carbon atom, and exist therefore as stereoisomers, including enantiomers and, when appropriate, diastereomers. The present invention includes the aforementioned stereoisomers within its scope and each of the individual *R* and *S* enantiomers of the compounds of formula I and their salts, substantially free, i.e. associated with less than 5%, preferably less than 2%, in particular less than 1% of the other enantiomer, and mixtures of such enantiomers in any proportions including the racemic mixtures containing substantially equal amounts or the two enantiomers.

Preferred are the α-amino acid phenyl ester derivatives of formula I wherein the configuration at the α-carbon atom is that of the *R*-enantiomer.
Particular preferred compounds according to the invention, which have found to be useful as hypnotics for intravenous anaesthesia, are:
R-2-[N-bis(2-methoxyethyl)amino]butyric acid 2,6-dimethoxy-4-methylphenyl ester;
R-2-[N-bis(2-methoxyethyl)amino]butyric acid, 2,6-dimethoxyphenyl ester; and
pharmaceutically acceptable salts thereof.

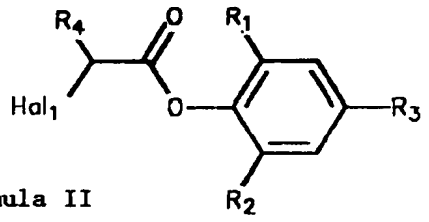
γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter within the central nervous system and it is probable that compounds potentiating the effects of GABA at $GABA_A$ receptors will induce anaesthesia (S. A. Zimmerman, M. V. Jon s and N. L.

Harrison, J. Pharmacol. Exp. Therap. 1994, 270, 987-991; N. P. Franks and W. R. Lieb, Nature 1994, 367, 607-614). Indeed there is compelling evidence that many hypnotics exert their biological activity via modulation of $GABA_A$ receptors, including steroids, barbiturates, benzodiazepines and propofol (D. L. Tanelian, P. Kosek, I. Mody and M. B. MacIver, Anesthesiology 1993, 78, 757-776). The compounds of the present invention have been shown to allosterically modulate $GABA_A$ receptors by inhibiting the specific binding of the radioligand [^{35}S]-*tert*-butyl bicyclophosphorothionate to rat whole brain membranes. The *in vitro* results presented in Table 1 demonstrate modulation of GABAergic function by the compounds of the present invention and suggest this mechanism mediates or enhances their hypnotic activity.

In addition to their general anaesthetic activity, the compounds of the invention can be used as sedative and analgesic drugs and in the treatment of GABA related diseases, such as anxiety (e.g. panic attack), stress, sleep disorders, post natal depression, and premenstrual tension, and in the alleviation of seizure.

The invention also relates to pharmaceutical compositions comprising an α-amino acid phenyl ester derivative having the general formula I or a pharmaceutically acceptable salt thereof.

The compounds of the invention may be prepared by condensation of an appropriately R_1,R_2,R_3-substituted phenyl, wherein R_1, R_2 and R_3 have the previously given meanings, with an acid halogenide according to the formula Hal_1-CHR_4-CO-Hal_2, wherein R_4 has the meaning as previously defined and Hal_1 and Hal_2 are independently iodo, bromo or chloro, preferably bromo, after which the resulting intermediate ester derivative of formula II



Formula II

is reacted with an amine according to the formula R_5R_6NH, wherein R_5 and R_6 have the meanings as previously defined, optionally followed by conversion into a pharmaceutically acceptable salt.

Alternatively the intermediate ester derivative of formula II may be prepared by condensation of an appropriately R_1,R_2,R_3-substituted phenyl, wherein R_1, R_2 and R_3 have the previously given meanings, with an acid according to the formula Hal_1-CHR_4-CO_2H, wherein R_4 has the meaning as previously defined and Hal_1 is iodo, bromo or chloro, preferably bromo, with the aid of a condensing agent, such as bromo-trispyrrolidino-phosphonium hexafluorophosphate (PyBrop), dicyclohexylcarbodiimide/N-hydroxybenzo-triazole and the like.

The compounds of the invention may also be prepared by condensation of an appropriately R_1,R_2,R_3-substituted phenyl, wherein R_1, R_2 and R_3 have the previously given meanings, with an α-amino acid derivative according to the formula R_5R_6N-CHR_4-CO_2H, wherein R_4, R_5 and R_6 have the previously given meanings, with the use of a condensation agent, such as those mentioned above.

The α-amino acid phenyl ester derivatives of Formula I contain at least one chiral carbon atom, i.e. the α-carbon atom. The compounds can therefore be obtained as pure stereoisomers, or as a mixture of stereoisomers. Methods for asymmetric synthesis whereby the pure stereoisomers are obtained are well known in the art, e.g. synthesis with chiral induction, enantioselective enzymatic ester hydrolysis, separation of stereoisomers or enantiomers using chromatography on chiral media. Such methods are for example described in *Chirality in Industry* (edited by A. N. Collins, G. N. Sheldrake and J. Crosby, 1992 ;John Wiley).

Pharmaceutically acceptable salts may be obtained by treating the free base of the compounds according to formula I with a mineral acid such as hydrochloric acid, phosphoric acid, sulphuric acid, preferably hydrochloric acid, or with an organic acid such as for example ascorbic acid, citric acid, tartaric acid, lactic acid, maleic acid, malonic acid,

fumaric acid, glycolic acid, succinic acid, propionic acid, acetic acid, m thanesulphonic acid and the like.

The present invention further provides pharmaceutical compositions comprising an α-amino acid phenyl ester derivative having the general formula I, or a pharmaceutically acceptable salt thereof, in admixture with pharmaceutically acceptable auxiliaries, and optionally other therapeutic agents. The term "acceptable" means being compatible with the other ingredients of the composition and not deleterious to the recipients thereof. Compositions include e.g. those suitable for oral, sublingual, subcutaneous, intravenous, intramuscular, local, or rectal administration, and the like, all in unit dosage forms for administration.

For oral administration, the active ingredient may be presented as discrete units, such as tablets, capsules, powders, granulates, solutions, suspensions, and the like.

For parenteral administration, the pharmaceutical composition of the invention may be presented in unit-dose or multi-dose containers, e.g. injection liquids in predetermined amounts, for example in sealed vials and ampoules, and may also be stored in a freeze dried (lyophilized) condition requiring only the addition of sterile liquid carrier, e.g. water, prior to use.

Mixed with such pharmaceutically acceptable auxiliaries, e.g. as described in the standard reference, Gennaro et al., Remington's Pharmaceutical Sciences, (18th ed., Mack Publishing Company, 1990, see especially Part 8: Pharmaceutical Preparations and Their Manufacture), the active agent may be compressed into solid dosage units, such as pills, tablets, or be processed into capsules or suppositories. By means of pharmaceutically acceptable liquids the active agent can be applied as a fluid composition, e.g. as an injection preparation, in the form of a solution, suspension, emulsion, or as a spray, e.g. a nasal spray.

For making solid dosage units, the use of conventional additives such as fillers, colorants, polymeric binders and the like is contemplated. In general any pharmaceutically acceptable additive which does not interfere with the function of the active compounds can be used. Suitable carriers with which the active agent of the invention can be administered as solid compositions include lactose, starch, cellulose derivatives and the lik , or mixtures thereof, used in suitable amounts. For parenteral administration, aqueous suspensions,

isoton saline solutions and st ril injectabl solutions may be used, containing pharmaceutically acceptable dispersing agents and/or wetting agents, such as propylene glycol or butylene glycol.

The invention further includes a pharmaceutical composition, as hereinbefore described, in combination with packaging material suitable for said composition, said packaging material including instructions for the use of the composition for the use as hereinbefore described.

The compounds of the invention may be administered for humans in a dosage of 0.001-50 mg per kg body weight, preferably in a dosage of 0.1-20 mg per kg body weight.

The invention is illustrated by the following examples.

EXAMPLES

Example 1.

a: (±)-2-bromobutyric acid, 2,6-dimethoxy-4-methylphenyl ester.

2-Bromobutyryl bromide (31.1 ml) was added to a stirred solution of 2,6-dimethoxy-4-methylphenol (45 g) in dry dichloromethane (500 ml), whereupon triethylamine (37.3 ml) was added dropwise over 30 minutes, maintaining the internal temperature below 10 °C using an ice-salt bath. During the addition a white precipitate formed. After addition was complete the reaction mixture was stirred for 1.5 hours, then filtered. The solid was washed with diethyl ether (200 ml) and the filtrate washed twice with saturated sodium bicarbonate solution (100 ml). The organic phase was dried over magnesium sulphate, filtered and the solvent removed under reduced pressure to give the crude product as an oil (79.4 g). To remove any residual starting phenol, the oil was dissolved in diethyl ether and washed with sodium hydroxide solution (0.1 M; 3 x 100 ml), then water (4 x 100 ml). The organic phase was dried over magnesium sulphate, filtered and the solvent removed under reduced pressure to give the title compound as a yellow oil (72.9 g).

^{1}H NMR ($CDCl_3$); δ 1.15 (t, 3H), 2.05-2.35 (m, 2H), 2.34 (s, 3H), 3.80 (s, 6H), 4.45 (t,1H), 6.40 (s, 2H).

The following compounds were prepared in a similar manner:

b: (±)-2-bromobutyric acid, 2,6-dimethoxyphenyl ester.

^{1}H NMR ($CDCl_3$); δ 1.15 (t, 3H), 2.10-2.35 (m, 2H), 3.82 (s, 6H), 4.47 (t, 1H), 6.60 (d, 2H), 7.15 (t, 1H).

c: (±)-2-bromopropionic acid, 2,6-dimethoxy-4-methylphenyl ester.

^{1}H NMR ($CDCl_3$); δ 1.97 (d, 3H), 2.35 (s, 3H), 3.80 (s, 6H), 4.68 (q, 1H), 6.42 (s, 2H).

d: (±)-2-bromopropionic acid, 2,6-dimethoxyphenyl ester

^{1}H NMR ($CDCl_3$); δ 1.98 (d, 3H), 3.80 (s, 3H), 4.68 (q, 1H), 6.61 (d, 2H), 7.14 (t,1H).

Example 2.

a: *S*-2-bromopropionic acid, 2,6-dimethoxyphenyl ester.

A solution of *S*-(-)-2-bromopropionic acid (58.8 g) in dry dichloromethane (590 ml) was stirred at room temperature. Oxalyl chloride (73 ml) and dichloromethane (70 ml) were added, after which gas evolution was observed. After 28 hours the solution was concentrated under reduced pressure and purged with dichloromethane (2 x 150 ml). Concentration of this solution (600 mmHg, 40 °C) gave a mixture of *S*-2-bromopropionyl chloride in dichloromethane [103 g, comprising *S*-2-bromopropionyl chloride (~76 g) and dichloromethane (~27 g)].

^{1}H NMR ($CDCl_3$); δ 1.92 (d, 3H), 4.65 (q, 1H).

A solution of *S*-2-bromopropionyl chloride (66 g) and 2,6-dimethoxyphenol (55 g) in dry toluene was stirred under nitrogen and cooled to -10 °C. A solution of dry pyridine (32.2 ml) in dry toluene (60 ml) was added dropwise keeping the temperature below 0 °C. After 20 minutes the resulting suspension was diluted with water (500 ml) and the mixture filtered through a dicalite pad to remove a small amount of white solid. The dicalite pad was rinsed with toluene (400 ml) and the filtrate was washed with water (3 x 150 ml) then dried over magnesium sulphate and filtered. The solution was concentrated under reduced pressure and purged with toluene to give *S*-2-bromopropionic acid, 2,6-dimethoxyphenyl ester (92.6 g) as a straw coloured oil which solidified on cooling. This material was sufficiently pure for use in subsequent steps. ^{1}H NMR and chiral analytical chromatography on a

Chiracel OJ column using hexane-isopropanol (9:1) as the eluent showed the product mixture comprised *S*-2-bromopropionic acid, 2,6-dimethoxyphenyl ester (91.3%), *R*-2-bromopropionic acid, 2,6-dimethoxyphenyl ester (4.8%) and *R*-2-chloropropionic acid, 2,6-dimethoxyphenyl ester (3.8%).

^{1}H NMR ($CDCl_3$); δ 1.98 (d, 3H), 3.82 (s, 3H), 4.70 (q, 1H), 6.65 (d, 2H), 7.15 (t,1H).

The following compound was prepared in a similar manner:

b: *S*-2-bromopropionic acid, 2,6-dimethoxy-4-methylphenyl ester.

^{1}H NMR ($CDCl_3$); δ 1.96 (d, 3H), 2.34 (s, 3H), 3.80 (s, 6H), 4.68 (q, 1H), 6.42 (s, 2H).

Example 3.

a: (±)-2-[N-bis(2-methoxyethyl)amino]butyric acid, 2,6-dimethoxy-4-methylphenyl ester.

A solution of 2-bromobutyric acid, 2,6-dimethoxy-4-methylphenyl ester (64.7 g) in dry toluene (328 ml) was heated to reflux with stirring, whereupon dry triethylamine (4 x 31.2 ml) and bis(2-methoxyethyl)amine (4 x 32.9 ml) were added as aliquots over 48 hours. The reaction mixture was allowed to cool, then filtered and the solid was washed with diethyl ether. The filtrate was concentrated under reduced pressure to low volume, then diluted with water (500 ml) and extracted with diethyl ether (3 x 350 ml). the combined extracts were washed with water (2 x 350 ml), then extracted with aqueous hydrochloric acid (1 M; 3 x 350 ml). The combined acidic extracts were cooled in ice-water and basified to pH 10 with sodium hydroxide solution (4M; 225 ml). The resulting solution was extracted with diethyl ether (3 x 500 ml) and the combined extracts washed with water (2 x 500 ml). The organic phase was dried over magnesium sulphate, filtered and the solvent removed under reduced pressure to give the crude product as an oil (50.5 g). Chromatography of this oil on silica gel and removal of any residual starting phenol as described above afforded th racemic titl compound as a yellow oil (46.6 g).

^{1}H NMR ($CDCl_3$); δ 1.05 (t, 3H), 1.65-1.8 (m, 1H), 1.9-2.05 (m, 1H), 2.35 (s, 3H), 2.85-3.1 (m, 4H), 3.36 (s, 6H), 3.4-3.5 (m, 4H), 3.55 (t, 1H), 3.77 (s, 6H), 6.40 (s, 2H). Positive ion ESI $(M+H)^+$ 370

The following compounds were prepared in a similar manner. In some instances acetone was used instead of toluene as the reaction solvent and diisopropylethylamine was used instead of triethylamine as a base. In several instances crude product mixtures were purified by chromatography on alumina rather than silica gel. Racemates are denoted (±), enantiomers (≥95% ee, resolved via chiral hplc or enzymatic methodology) are denoted by absolute stereochemistry i.e. *R* or *S* and/or optical rotation i.e. (+) or (-). while enantiomeric mixtures (<97% ee, prepared from the above *S*-bromo phenolic esters) have no stereochemistry assigned, i.e. there is no (+), (-), (+/-), R or S prior to the chemical name (e.g. example 7i).

b: (±)-2-[N-bis(2-methoxyethyl)amino]butyric acid, 2,6-dimethoxyphenyl ester.

^{1}H NMR ($CDCl_3$); δ 1.05 (t, 3H), 1.65-2.05 (m, 2H), 2.8-3.15 (m, 4H), 3.3-3.65 (m, 5H), 3.36 (s, 6H), 3.80 (s, 6H), 6.6 (d, 2H), 7.15 (t, 1H).

c: (±)-2-[N-bis(2-ethoxyethyl)amino]propionic acid, 2,6-dimethoxyphenyl ester.

^{1}H NMR ($CDCl_3$); δ 1.20 (t, 6H), 1.48 (d, 3H), 2.8-3.1 (m, 4H), 3.45-3.65 (m, 8H), 3.80 (s, 6H), 3.90 (q, 1H), 6.6 (d, 2H), 7.1 (t, 1H). IR (thin film): 1758, 1607, 1482, 1304, 1260, 1115 cm^{-1}. Positive ion ESI $(M+H)^+$ 370

d: (±)-2-[N-bis(2-methoxyethyl)amino]propionic acid, 2,6-dimethoxy-4-methylphenyl ester.

^{1}H NMR ($CDCl_3$); δ 1.5 (d, 3H), 2.35 (s, 3H), 2.85-3.15 (m, 4H), 3.35 (s, 6H), 3.4-3.55 (m, 4H), 3.8 (s, 6H), 3.9 (q, 1H), 6.4 (s, 2H).

e: (±)-2-[N-bis(2-methoxyethyl)amino]propionic acid, 2,6-dimethoxyphenyl ester.

^{1}H NMR ($CDCl_3$); δ 1.50 (d, 3H), 2.85-3.15 (m, 4H), 3.36 (s, 6H), 3.4-3.6 (m, 4H), 3.80 (s, 6H), 3.90 (q, 1H), 6.6 (d, 2H), 7.1 (t, 1H).

f: (±)-2-[N-methylbenzylamino]propionic acid, 2,6-dimethoxy-4-methylphenyl ster.

Positiv ion ESI $(M+H)^+$ 344

g: 2-[N-m thylbenzylamino]propionic acid, 2,6-dimethoxy-4-methylphenyl ester.
Positiv ion ESI $(M+H)^+$ 344

h: 2-[N-methylallylamino]propionic acid, 2,6-dimethoxy-4-methylphenyl ester.
Positive ion ESI $(M+H)^+$ 294

i: (±)-2-[diethylamino]propionic acid, 2,6-dimethoxy-4-methylphenyl ester.
Positive ion ESI $(M+H)^+$ 296

j: (±)-2-[N-methylbenzylamino]butyric acid, 2,6-dimethoxyphenyl ester.
^1H NMR ($CDCl_3$); δ 1.1 (t, 3H), 1.75-2.1 (m, 2H), 2.40 (s, 3H), 3.55 (t, 1H), 3.7-4.0 (m, 2H), 3.83 (s, 6H), 6.65 (d, 2H), 7.1-7.45 (m, 6H). Positive ion ESI $(M+H)^+$ 344

k: (±)-2-[N-bis(2-ethoxyethyl)amino]butyric acid, 2,6-dimethoxyphenyl ester.
Positive ion ESI $(M+H)^+$ 384

l: 2-[N-methylphenethylamino]propionic acid, 2,6-dimethoxyphenyl ester.
Positive ion ESI $(M+H)^+$ 344

m: (±)-2-[N-bis(2-methoxyethyl)amino]butyric acid, 2,6-diethoxyphenyl ester
Positive ion ESI $(M+H)^+$ 384

n: (±)-2-[N-bis(2-methoxyethyl)amino]butyric acid, 2,6-di-(1-methyl)ethoxyphenyl ester.
Positive ion ESI $(M+H)^+$ 412

o: 2-[N-methyl-(2-methoxy)ethylamino]propionic acid, 2,6-dimethoxyphenyl ester.
Positive ion ESI $(M+H)^+$ 312

p: (±)-2-[N-bis(2-methoxycarboylethyl)amino]butyric acid, 2,6-dimethoxyphenyl ester. Positive ion ESI $(M+H)^+$ 412

q: (±)-2-[N-(2-ethoxycarbonylethyl)amino-N-(2-methoxycarbonylethyl)amino]butyric acid, 2,6-dimethoxyphenyl ester. Positive ion ESI $(M+H)^+$ 426

Example 4.

a: (±)-2-[N-bis(2-methoxyethyl)amino]pentanoic acid, 2,6-dimethoxyphenyl ester.

To a stirred solution of (±)-2-[N-bis(2-methoxyethyl)amino]pentanoic acid hydrochloride (1:1) salt (14 g) in dimethylformamide (280 ml) was added triethylamine (7.3 ml). After 30 minutes 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (12.92 g) was added. After a further 30 minut s 2,6-dimethoxyphenol

(7.99 g) and N,N-dimethylaminopyridine (189 mg) were added and stirring continued for 3 days. The reaction mixture was poured into water, extracted with dichloromethane and the combined extracts washed with dilute hydrochloric acid, dried over sodium sulphate, filtered and the solvent removed under reduced pressure to give the crude product as an oil (10.24 g). Chromatography of this oil on alumina afforded the racemic title compound as an oil (1.48 g).

^{1}H NMR ($CDCl_3$); δ 1.0 (t, 3H), 1.45-1.6 (m, 2H), 1.65-1.75 (m, 1H), 1.85-1.95 (m, 1H), 2.9-3.1 (m, 4H), 3.36 (s, 6H), 3.4-3.6 (m, 4H), 3.7 (t, 1H), 3.80 (s, 6H), 6.6 (d, 2H), 7.1 (t, 1H).

Positive ion ESI $(M+H)^+$ 344

The following compounds were prepared in a similar manner:

b: (±)-2-[N-bis(2-methoxyethyl)amino]butyric acid, 2,4,6-trimethoxyphenyl ester.
Positive ion ESI $(M+H)^+$ 386

c:(±)-3-methyl-2-[N-bis(2-methoxyethyl)amino]butyric acid, 2,6-dimethoxyphenyl ester. Positive ion ESI $(M+H)^+$ 370

Example 5.

a: *R*-(+)-2-[N-bis(2-methoxyethyl)amino]butyric acid, 2,6-dimethoxy-4-methylphenyl ester.

The racemic 2-[N-bis(2-methoxyethyl)amino]butyric acid, 2,6-dimethoxy-4-methylphenyl ester, described previously, was resolved via chiral preparative chromatography on a Chiracel OJ column (2 cm x 25 cm; Daicel) using hexane-isopropanol-diethylamine (95:5:0.1 v/v/v) as the eluent. The title compound with *R* absolute configuration eluted first; $[\alpha]_D$ = +43.3 ° (c=0.6 in chloroform).

The following compounds were prepared in a similar manner:

b: *S*-(-)-2-[N-bis(2-methoxyethyl)amino]butyric acid, 2,6-dimethoxy-4-methylph nyl ester. Positive ion ESI $(M+H)^+$ 370

c: *R*-2-[N-bis(2-ethoxyethyl)amino]propionic acid, 2,6-dimethoxyphenyl ester. Positive ion ESI $(M+H)^+$ 370

d: *S*-2-[N-bis(2-ethoxyethyl)amino]propionic acid, 2,6-dimethoxyphenyl ester. Positive ion ESI $(M+H)^+$ 370

e: *R*-2-[N-bis(2-methoxyethyl)amino]propionic acid, 2,6-dimethoxy-4-methylphenyl ester. Positive ion ESI $(M+H)^+$ 356

f: *R*-2-[N-bis(2-ethoxyethyl)amino]butyric acid, 2,6-dimethoxyphenyl ester. Positive ion ESI $(M+H)^+$ 384

g: *S*-2-[N-bis(2-ethoxyethyl)amino]butyric acid, 2,6-dimethoxyphenyl ester. Positive ion ESI $(M+H)^+$ 384

Example 6.

a: *R*-2-[N-bis(2-methoxyethyl)amino]butyric acid, 2,6-dimethoxyphenyl ester.

(±)-2-[N-bis(2-methoxyethyl)amino]butyric acid, 2,6-dimethoxyphenyl ester (40.0 g) was dissolved in phosphate buffer (1066 mL; prepared with disodium hydrogen phosphate (17.32 g) and sodium dihydrogen phosphate dihydrate (12.17 g) per litre of water and the pH adjusted to 7.0 with 2M sodium hydroxide solution). Porcine liver esterase (5.27 g, 19 units/mg solid, Sigma cat. no. E3019) was added to this mixture, which was stirred for 4 days at room temperature. Methyl t-butyl ether (1 l) was then added and the mixture stirred overnight. The layers were separated and the aqueous phase extracted again with methyl t-butyl ether (1 l). The combined organic liquors were dried over sodium sulphate, filtered and the solvent removed under reduced pressure to give the crude product as an oil (20.5 g). Chromatography of this oil on alumina using petroleum ether-ethyl acetate (7:3 v/v) as the eluent afforded the title compound as an oil (10.14 g).
Positive ion ESI $(M+H)^+$ 356

The following compound was prepared in a similar manner:

b: *R*-2-[N-bis(2-methoxyethyl)amino]pentanoic acid, 2,6-dimethoxyphenyl ester.
Positive ion ESI $(M+H)^+$ 370

Example 7.

a: *R*-(+)-2-[N-bis(2-methoxyethyl)amino]butyric acid, 2,6-dimethoxy-4-methylphenyl ester hydrochloride (1:1) salt.

Hydrogen chloride gas was passed through a solution of 2*R*-[N-bis(2-methoxyethyl)amino]butyric acid, 2,6-dimethoxy-4-methylphenyl ester (16.5 g) in diethylether (175 ml) for 2-3 minutes, after which precipitation of the salt was deemed complete. The solvent was removed under reduced pressure to give a gummy solid which was suspended in a mixture of diethyl ether (120 ml) and dichloromethane (20 ml). This mixture was stirred rapidly and cooled using an ice bath. The resulting white solid that precipitated was filtered off and washed with diethyl ether to give the title compound (14.5 g).

^{1}H NMR ($CDCl_3$ + C_5D_5N); δ 1.13 (t, 3H), 1.95-2.1 (m, 2H), 2.34 (s, 3H), 3.05-3.35 (m, 4H), 3.37 (s, 6H), 3.55-3.75 (m, 4H), 3.78 (s, 6H), 3.85 (t, 1H), 6.3 (br, NH^+), 6.40 (s, 2H). IR (KBr disc): 3416, 1768, 1606, 1506, 1465 cm^{-1}.

$[\alpha]_D$ = +7.03 ° (c=0.8 in chloroform).

The following compounds were prepared in a similar manner. In some cases the salt was prepared and isolated without dichloromethane.

b: (±)-2-[N-bis(2-methoxyethyl)amino]butyric acid, 2,6-dimethoxy-4-methylphenyl ester hydrochloride (1:1) salt.

^{1}H NMR ($CDCl_3$ + C_5D_5N); δ 1.31 (t, 3H), 1.95-2.15 (m, 2H), 2.34 (s, 3H), 3.1-3.35 (m, 4H), 3.37 (s, 6H), 3.55-3.65 (m, 2H), 3.7-3.8 (m, 2H), 3.78 (s, 6H), 3.85 (q, 1H), 6.42 (s, 2H), 6.9 (br, NH^+). IR (KBr disc): 1768, 1607, 1508, 1470, 1412 cm^{-1}.

Elemental Analysis: For $C_{19}H_{32}NO_6Cl$ $0.125H_2O$. Calcd: C, 55.91; H, 7.96; N, 3.43; Cl, 8.69. Found: C, 55.89; H, 7.78; N, 3.37; Cl, 8.79.

c: (±)-2-[N-bis(2-methoxyethyl)amino]butyric acid, 2,6-dim thoxyphenyl est r hydrochloride (1:1) salt.

^{1}H NMR ($CDCl_3$ + C_5D_5N); δ 1.13 (t, 3H), 1.95-2.05 (m, 2H), 3.05-3.3 (m, 4H), 3.38 (s, 6H), 3.55-3.75 (m, 4H), 3.80 (s, 6H), 3.85 (t, 1H), 5.65 (br, NH^+), 6.6 (d, 2H), 7.14 (t, 1H). IR (KBr disc): 3507, 3427, 2531, 1762, 1606, 1585, 1500, 1483, 1462, 1439 cm^{-1}. Elemental Analysis: For $C_{18}H_{30}NO_6Cl$ 1.0H_2O. Calcd: C, 52.74; H, 7.87; N, 3.42; Cl, 8.65. Found: C, 52.90; H, 7.80; N, 3.47; Cl, 8.64.

d: (±)-2-[N-bis(2-ethoxyethyl)amino]propionic acid, 2,6-dimethoxyphenyl ester hydrochloride (1:1) salt.

^{1}H NMR ($CDCl_3$ + C_5D_5N); δ 1.21 (t, 6H), 1.71 (d, 3H), 3.2-3.4 (m, 4H), 3.5-3.6 (m, 4H), 3.6-3.75 (m, 2H), 3.80 (s, 6H), 3.8-3.9 (m, 2H), 4.35 (q, 1H), 5.9 (br, NH^+), 6.60 (d, 2H), 7.15 (t, 1H). IR (KBr disc): 1768, 1606, 1585, 1484, 1454 cm^{-1}.
Elemental Analysis: For $C_{19}H_{32}NO_6Cl$. Calcd: C, 56.22; H, 7.95; N, 3.45; Cl, 8.73. Found: C, 56.15; H, 7.73; N, 3.44; Cl, 8.79.

e: (±)-2-[N-bis(2-methoxyethyl)amino]propionic acid, 2,6-dimethoxy-4-methylphenyl ester hydrochloride (1:1) salt.

M.p. 96-97°C; ^{1}H NMR ($CDCl_3$ + C_5D_5N); δ 1.67 (d, 3H), 2.33 (s, 3H), 3.15-3.35 (m, 4H), 3.37 (s, 6H), 3.55-3.65 (m, 2H), 3.7-3.85 (m, 2H), 3.77 (s, 6H), 4.25 (q, 1H), 6.41 (s, 2H), 6.7 (br, NH^+). IR (KBr disc): 3461, 2412, 1771, 1604, 1510, 1472, 1413 cm^{-1}. Elemental Analysis: For $C_{18}H_{29}NO_6$ 1.2HCl 0.375H_2O. Calcd: C, 53.26; H, 7.69; N, 3.45; Cl, 10.48. Found: C, 53.43; H, 7.49; N, 3.46; Cl, 10.47.

f: (±)-2-[N-bis(2-methoxyethyl)amino]propionic acid, 2,6-dimethoxyphenyl ester hydrochloride (1:1) salt.

M.p. 114-116°C; ^{1}H NMR ($CDCl_3$ + C_5D_5N); δ 1.69 (d, 3H), 3.15-3.35 (m, 4H), 3.38 (s, 6H), 3.55-3.65 (m, 2H), 3.7-3.85 (m, 2H), 3.80 (s, 6H), 4.25 (q, 1H), 6.5 (br, NH^+), 6.60 (d, 2H), 7.14 (t, 1H). IR (KBr disc): 3405, 2405, 2211, 1762, 1618, 1605, 1585, 1485, 1464, 1454 cm^{-1}. Elemental Analysis: For $C_{17}H_{27}NO_6$ 1.05HCl 0.125H_2O. Calcd: C, 53.46; H, 7.47; N, 3.67; Cl, 9.75. Found: C, 53.64; H, 7.41; N, 3.63; Cl, 9.85.

g: (±)-2-[N-methylbenzylamino]propionic acid, 2,6-dimethoxy-4-methylphenyl ester hydrochloride (1:1) salt.

^{1}H NMR ($CDCl_3$ + C_5D_5N); δ 1.80 (d, 3H), 2.37 (s, 3H), 2.81 (s, 3H), 3.82 (s, 6H), 4.10 (q, 1H), 4.2-4.4 (m, 2H), 6.45 (s, 2H), 7.35-7.45 (m, 3H), 7.65-7.75 (m, 2H).
IR (KBr disc): 2335, 1764, 1610, 1510, 1471, 1411 cm^{-1}.
Elemental Analysis: For $C_{20}H_{26}NO_4Cl$. Calcd: C, 63.24; H, 6.90; N, 3.69; Cl, 9.33. Found: C, 63.11; H, 6.71; N, 3.43; Cl, 8.99.

h: *R*-(+)-2-[N-methylbenzylamino]propionic acid, 2,6-dimethoxy-4-methylphenyl ester hydrochloride (1:1) salt.

^{1}H NMR ($CDCl_3$ + C_5D_5N); δ 1.80 (d, 3H), 2.37 (s, 3H), 2.81 (s, 3H), 3.82 (s, 6H), 4.10 (q, 1H), 4.2-4.4 (m, 2H), 6.45 (s, 2H), 7.35-7.45 (m, 3H), 7.65-7.75 (m, 2H).
IR (KBr disc): 2323, 1758, 1605, 1497, 1467, 1415 cm^{-1}.
Elemental Analysis: For $C_{20}H_{26}NO_4Cl$. Calcd: C, 63.24; H, 6.90; N, 3.69; Cl, 9.33. Found: C, 63.00; H, 6.79; N, 3.69; Cl, 8.85. $[\alpha]_D$ = +67.8 ° (c=0.8 in chloroform)

i: 2-[N-methylallylamino]propionic acid, 2,6-dimethoxy-4-methylphenyl ester hydrochloride (1:1) salt.

^{1}H NMR ($CDCl_3$ + C_5D_5N); δ 1.88 (d, 3H), 2.36 (s, 3H), 2.89 (s, 3H), 3.75-3.90 (m, 2H), 3.80 (s, 6H), 4.35 (q, 1H), 5.45-5.55 (m, 2H) 6.25-6.40 (m, 1H), 6.45 (s, 2H).
IR (KBr disc): 2323, 1758, 1605, 1497, 1467, 1415 cm^{-1}.
Elemental Analysis: For $C_{16}H_{24}NO_4Cl$ 0.4H_2O. Calcd: C, 57.02; H, 7.42; N, 4.16; Cl, 10.52. Found: C, 57.05; H, 7.20; N, 4.21; Cl, 10.16.

j: (±)-2-[diethylamino]propionic acid, 2,6-dimethoxy-4-methylphenyl ester hydrochloride (1:1) salt.

^{1}H NMR ($CDCl_3$ + C_5D_5N); δ 1.52 (t, 6H), 1.92 (d, 3H), 2.35 (s, 3H), 3.15-3.30 (m, 2H), 3.45-3.60 (m, 2H), 3.78 (s, 6H), 4.45 (q, 1H), 6.40 (s, 2H). IR (KBr disc): 2265, 1760, 1613, 1513, 1468, 1418 cm^{-1}. Elemental Analysis: For $C_{16}H_{26}NO_4Cl$ 0.125H_2O. Calcd: C, 57.52; H, 7.92; N, 4.19; Cl, 10.61. Found: C, 57.53; H, 7.91; N, 4.26; Cl, 10.89.

k: (±)-2-[N-methylbenzylamino]butyric acid, 2,6-dimethoxyphenyl ester hydrochloride (1:1) salt.

^{1}H NMR ($CDCl_3$ + C_5D_5N); δ 1.16 (t, 3H), 2.00-2.15 (m, 1H), 2.20-2.35 (m, 1H), 2.71 (s, 3H), 3.8-3.9 (m, 1H), 3.85 (s, 6H), 4.15-4.35 (m, 2H), 5.2 (br, NH^+), 6.65 (d, 2H), 7.2 (t, 1H), 7.3-7.45 (m, 3H), 7.6-7.7 (m, 2H). IR (KBr disc): 3499, 2418, 2355, 1759,

1610, 1585, 1499, 1484, 1460 cm^{-1}. Elemental Analysis: For $C_{20}H_{26}NO_4Cl$ $0.25H_2O$. Calcd: C, 62.49; H, 6.95; N, 3.64; Cl, 9.22. Found: C, 62.52; H, 6.85; N, 3.61; Cl, 9.58.

l: (±)-2-[N-bis(2-ethoxyethyl)amino]butyric acid, 2,6-dimethoxyphenyl ester hydrochloride (1:1) salt.

^1H NMR ($CDCl_3$ + C_5D_5N); δ 1.12 (t, 3H), 1.20 (t, 6H), 1.95-2.10 (m, 2H), 3.1-3.3 (m, 4H), 3.52 (q, 4H), 3.55-3.75 (m, 4H), 3.80 (s, 6H), 3.85 (t, 1H), 6.60 (d, 2H), 7.13 (t, 1H), 7.4 (br, NH^+). IR (KBr disc): 3431, 2282, 1766, 1612, 1587, 1482, 1459, 1441 cm^{-1}. Elemental Analysis: For $C_{20}H_{34}NO_6Cl$. Calcd: C, 57.20; H, 8.16; N, 3.34; Cl, 8.44. Found: C, 56.81; H, 8.18; N, 3.34; Cl, 8.60.

m: 2-[N-methylphenethylamino]propionic acid, 2,6-dimethoxyphenyl ester hydrochloride (1:1) salt.

^1H NMR ($CDCl_3$ + C_5D_5N); δ 1.87 (d, 3H), 2.94 (s, 3H), 3.2-3.45 (m, 4H), 3.76 (s, 6H), 4.35 (q, 1H), 6.62 (d, 2H), 7.19 (t, 1H), 7.2-7.35 (m, 5H). IR (KBr disc): 3439, 2412, 1774, 1605, 1585, 1498, 1482, 1457 cm^{-1}. Elemental Analysis: For $C_{20}H_{26}NO_4Cl$. Calcd: C, 63.24; H, 6.90; N, 3.69; Cl, 9.33. Found: C, 63.13; H, 6.86; N, 3.85; Cl, 9.38. $[\alpha]_D$ = +3.3 ° (c=0.6 in chloroform)

n: (±)-2-[N-bis(2-methoxyethyl)amino]butyric acid, 2,6-diethoxyphenyl ester hydrochloride (1:1) salt.

^1H NMR ($CDCl_3$ + C_5D_5N); δ 1.15 (t, 3H), 1.36 (t, 6H), 1.95-2.05 (m, 2H), 3.05-3.30 (m, 4H), 3.37 (s, 6H), 3.55-3.75 (m, 4H), 3.8 (m. 1H), 3.85-4.1 (m, 4H), 6.56 (d, 2H), 6.7 (br, NH^+), 7.1 (t, 1H). IR (KBr disc): 2266, 1763, 1603, 1581, 1499, 1475, 1400 cm^{-1}. Elemental Analysis: For $C_{20}H_{34}NO_6Cl$. Calcd: Cl, 8.04. Found: Cl, 8.44.

o: (±)-2-[N-bis(2-methoxyethyl)amino]butyric acid, 2,6-di-(1-methyl)ethoxyphenyl ester hydrochloride (1:1) salt.

^1H NMR ($CDCl_3$ + C_5D_5N); δ 1.14 (t, 3H), 1.20-1.35 (m, 12H), 1.95-2.05 (m, 2H), 3.05-3.30 (m, 4H), 3.37 (s, 6H), 3.5-3.7 (m, 4H), 3.8 (t, 1H), 4.45-4.60 (m, 2H), 5.7 (br, NH^+), 6.55 (d, 2H), 7.06 (t, 1H). IR (KBr disc): 2374, 1759, 1607, 1579, 1476 cm^1. Elemental Analysis: For $C_{22}H_{38}NO_6Cl$. Calcd: C, 58.98; H, 8.55; N, 3.13; Cl, 7.91. Found: C, 58.78; H, 8.51; N, 3.12; Cl, 7.32.

p: 2-[N-methyl-(2-m thoxy)ethylamino]propionic acid, 2,6-dim thoxyphenyl ster hydrochloride (1:1) salt.

^{1}H NMR ($CDCl_3$ + C_5D_5N); δ 1.83 (d, 3H), 2.35 (s, 3H), 2.93 (s, 3H), 3.3-3.45 (m, 2H), 3.40 (s, 3H), 3.79 (s, 6H), 3.8-4.0 (m, 2H), 4.35 (q, 1H), 6.43 (s, 2H).

IR (KBr disc): 2430, 1770, 1609, 1511, 1468, 1420 cm^{-1}. Elemental Analysis: For $C_{16}H_{26}NO_5Cl$. Calcd: C, 55.25; H, 7.53; N, 4.03; Cl, 10.19. Found: C, 55.23; H, 7.31; N, 4.06; Cl, 9.48. $[\alpha]_D$ = +11.4 ° (c=0.6 in chloroform)

q: (±)-2-[N-bis(2-methoxycarbonylethyl)amino]butyric acid, 2,6-dimethoxyphenyl ester hydrochloride (1:1) salt.

^{1}H NMR ($CDCl_3$ + C_5D_5N); δ 0.94 (t, 3H), 1.55-1.70 (m, 1H), 1.80-1.95 (m, 1H), 2.45 (t, 4H), 2.85-3.15 (m, 4H), 3.45 (t, 1H), 3.60 (s, 6H), 3.72 (s, 6H), 6.52 (d, 2H), 7.1 (t, 1H).

r: (±)-2-[N-(2-ethoxycarbonylethyl)amino-N-(2-methoxycarbonylethyl)amino]butyric acid, 2,6-dimethoxyphenyl ester hydrochloride (1:1) salt.

^{1}H NMR ($CDCl_3$ + C_5D_5N); δ 1.01 (t, 3H), 1.26 (t, 3H), 1.65-1.80 (m, 1H), 1.90-2.05 (m, 1H), 2.45-2.55 (m, 4H), 2.95-3.20 (m, 4H), 3.55 (t, 1H), 3.67 (s, 3H), 3.80 (s, 6H), 4.15 (q, 2H), 6.59 (d, 2H), 7.1 (t, 1H).

s: (±)-2-[N-bis(2-methoxyethyl)amino]pentanoic acid, 2,6-dimethoxyphenyl ester hydrochloride (1:1) salt.

^{1}H NMR ($CDCl_3$ + C_5D_5N); δ 1.0 (t, 3H), 1.5-1.65 (m, 2H), 1.85-2.0 (m, 2H), 3.05-3.3 (m, 4H), 3.37 (s, 6H), 3.5-3.7 (m, 4H), 3.80 (s, 6H), 3.9 (t, 1H), 6.6 (d, 2H), 7.13 (t, 1H), 7.2 (br, NH^+). IR (KBr disc): 2328, 2113, 1750, 1607, 1585, 1499, 1484 cm^{-1}. Elemental Analysis: For $C_{19}H_{32}NO_6Cl$. Calcd: C, 56.22; H, 7.95; N, 3.45; Cl, 8.73. Found: C, 56.20; H, 7.93; N, 3.46; Cl, 8.63.

t: (±)-2-[N-bis(2-methoxyethyl)amino]butyric acid, 2,4,6-trimethoxyphenyl ester hydrochloride (1:1) salt.

^{1}H NMR ($CDCl_3$ + C_5D_5N); δ 1.10 (t, 3H), 1.95-2.05 (m, 2H), 3.05-3.30 (m, 4H), 3.37 (s, 6H), 3.55-3.75 (m, 4H), 3.78 (s, 6H), 3.8 (m, 1H), 3.80 (s, 3H), 6.15 (s, 2H), 7.15 (br, NH^+). IR (KBr disc): 2155, 1752, 1611, 1595, 1507, 1464, 1434, 1420 cm^{-1}. Elemental Analysis: For $C_{19}H_{32}NO_7Cl$. Calcd: C, 54.09; H, 7.65; N, 3.32; Cl, 8.40. Found: C, 53.79; H, 7.66; N, 3.29; Cl, 8.12.

u: (±)-3-methyl-2-[N-bis(2-methoxyethyl)amino]butyric acid, 2,6-dimethoxyphenyl ester hydrochloride (1:1) salt.

^{1}H NMR ($CDCl_3$ + C_5D_5N); δ 1.0-1.1 (m, 6H), 2.05-2.2 (m, 1H), 2.85-3.10 (m, 4H), 3.20 (d, 1H), 3.36 (s, 6H), 3.40-3.55 (m, 4H), 3.79 (s, 6H), 6.4 (br, NH^+), 6.6 (d, 2H), 7.1 (t, 1H). IR (KBr disc): 3424, 2449, 1763, 1608, 1587, 1500, 1482, 1403 cm^{-1}.

v: *S*-(-)-2-[N-bis(2-methoxyethyl)amino]butyric acid, 2,6-dimethoxy-4-methylphenyl ester hydrochloride (1:1) salt.

^{1}H NMR ($CDCl_3$ + C_5D_5N); δ 1.13 (t, 3H), 1.95-2.1 (m, 2H), 2.33 (s, 3H), 3.05-3.35 (m, 4H), 3.36 (s, 6H), 3.55-3.75 (m, 4H), 3.75 (t, 1H), 3.77 (s, 6H), 5.2 (br, NH^+), 6.40 (s, 2H). IR (KBr disc): 3416, 2436, 1768, 1606, 1506, 1465 cm^{-1}.

Elemental Analysis: For $C_{19}H_{32}NO_6Cl$ $0.625H_2O$. Calcd: C, 54.70; H, 803; N, 3.36; Cl, 8.50. Found: C, 54.68; H, 7.81; N, 3.35; Cl, 8.44. $[\alpha]_D$ = -5.5 ° (c=0.7 in chloroform).

w: (+)-2-[N-bis(2-ethoxyethyl)amino]propionic acid, 2,6-dimethoxyphenyl ester hydrochloride (1:1) salt.

^{1}H NMR ($CDCl_3$ + C_5D_5N); δ 1.21 (t, 6H), 1.75 (d, 3H), 3.3-3.45 (m, 4H), 3.5-3.6 (m, 4H), 3.65-3.75 (m, 2H), 3.80 (s, 6H), 3.85-4.0 (m, 2H), 4.4 (q, 1H), 6.60 (d, 2H), 6.8 (br, NH^+), 7.15 (t, 1H). IR (KBr disc): 3425, 2462, 1765, 1617, 1586, 1498, 1482, 1457, 1439 cm^{-1}. Elemental Analysis: For $C_{19}H_{32}NO_6Cl$ $0.125H_2O$. Calcd: C, 55.91; H, 7.96; N, 3.43; Cl, 8.69. Found: C, 55.81; H, 7.92; N, 3.40; Cl, 8.88.

$[\alpha]_D$ = +6.6 ° (c=0.6 in chloroform)

x: (-)-2-[N-bis(2-ethoxyethyl)amino]propionic acid, 2,6-dimethoxyphenyl ester hydrochloride (1:1) salt.

^{1}H NMR ($CDCl_3$ + C_5D_5N); δ 1.23 (t, 6H), 1.78 (d, 3H), 3.3-3.45 (m, 4H), 3.5-3.6 (m, 4H), 3.65-3.75 (m, 2H), 3.82 (s, 6H), 3.85-4.0 (m, 2H), 4.45 (q, 1H), 6.65 (d, 2H), 6.7 (br, NH^+), 7.20 (t, 1H). IR (KBr disc): 3423, 2329, 1766, 1617, 1586, 1482, 1457 cm^{-1}

Elemental Analysis: For $C_{19}H_{32}NO_6Cl$ $0.25H_2O$. Calcd: C, 55.60; H, 7.98; N, 3.41; Cl, 8.64. Found: C, 55.63; H, 7.88; N, 3:35; Cl, 8.72. $[\alpha]_D$ = -4.9 ° (c=0.7 in chloroform)

y: *R*-(+)-2-[N-bis(2-methoxyethyl)amino]propionic acid, 2,6-dimethoxy-4-methyl-phenyl ester hydrochloride (1:1) salt.

^{1}H NMR ($CDCl_3$ + C_5D_5N); δ 1.67 (d, 3H), 2.33 (s, 3H), 3.15-3.35 (m, 4H), 3.37 (s, 6H), 3.55-3.65 (m, 2H), 3.7-3.85 (m, 2H), 3.78 (s, 6H), 4.25 (q, 1H), 6.41 (s, 2H), 6.5 (br, NH^+). IR (KBr disc): 3349, 2476, 1772, 1604, 1507, 1468, 1416 cm^{-1}.

Elemental Analysis: For $C_{18}H_{30}NO_6Cl$ $0.25H_2O$. Calcd: C, 54.54; H, 7.76; N, 3.53; Cl, 8.94. Found: C, 54.41; H, 7.70; N, 3.51; Cl, 8.91. $[\alpha]_D$ = +9.5 ° (c=0.3 in chloroform)

z: *R*-(+)-2-[N-bis(2-ethoxyethyl)amino]butyric acid, 2,6-dimethoxyphenyl ester hydrochloride (1:1) salt.

^{1}H NMR ($CDCl_3$ + C_5D_5N); δ 1.12 (t, 3H), 1.20 (t, 6H), 1.95-2.10 (m, 2H), 3.1-3.3 (m, 4H), 3.52 (q, 4H), 3.5-3.7 (m, 4H), 3.75 (t, 1H), 3.79 (s, 6H), 6.3 (br, NH^+) 6.60 (d, 2H), 7.13 (t, 1H). IR (KBr disc): 3460, 2131, 1755, 1610, 1587, 1484, 1462 cm^{-1}.

Elemental Analysis: For $C_{20}H_{34}NO_6Cl$. Calcd: C, 57.20; H, 8.16; N, 3.34; Cl, 8.44. Found: C, 57.07; H, 8.09; N, 3.27; Cl, 8.39. $[\alpha]_D$ = +5.2 ° (c=0.5 in chloroform)

aa: *S*-(-)-2-[N-bis(2-ethoxyethyl)amino]butyric acid, 2,6-dimethoxyphenyl ester hydrochloride (1:1) salt.

^{1}H NMR ($CDCl_3$ + C_5D_5N); δ 1.13 (t, 3H), 1.20 (t, 6H), 1.95-2.15 (m, 2H), 3.1-3.3 (m, 4H), 3.52 (q, 4H), 3.6-3.8 (m, 4H), 3.79 (s, 6H), 3.90 (t, 1H), 6.60 (d, 2H), 7.14 (t, 1H), 7.3 (br, NH^+). IR (KBr disc): 2121, 1754, 1610, 1587, 1485, 1462 cm^{-1}.

Elemental Analysis: For $C_{20}H_{34}NO_6Cl$. Calcd: C, 57.20; H, 8.16; N, 3.34; Cl, 8.44. Found: C, 57.07; H, 8.09; N, 3.27; Cl, 8.39. $[\alpha]_D$ = -3.0 ° (c=0.5 in chloroform)

ab: *R*-(+)-2-[N-bis(2-methoxyethyl)amino]butyric acid, 2,6-dimethoxyphenyl ester hydrochloride (1:1) salt.

^{1}H NMR ($CDCl_3$ + C_5D_5N); δ 1.12 (t, 3H), 1.90-2.05 (m, 2H), 3.0-3.25 (m, 4H), 3.37 (s, 6H), 3.55-3.70 (m, 4H), 3.80 (s, 6H), 3.75 (t, 1H), 5.25 (br, NH^+), 6.60 (d, 2H), 7.15 (t, 1H). IR (KBr disc): 3498, 2423, 1762, 1606, 1585, 1483, 1461 cm^{-1}.

$[\alpha]_D$ = +4.6 ° (c=0.5 in chloroform)

ac: *R*-2-[N-bis(2-methoxyethyl)amino]pentanoic acid, 2,6-dimethoxyphenyl ester hydrochloride (1:1) salt.

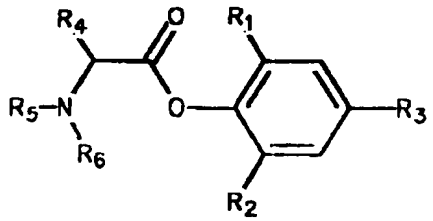
^{1}H NMR ($CDCl_3$ + C_5D_5N); δ 1.00 (t, 3H), 1.50-1.65 (m, 2H), 1.88-1.98 (m, 2H), 3.0-3.25 (m, 4H), 3.37 (s, 6H), 3.55-3.70 (m, 4H), 3.80 (s, 6H), 3.88 (t, 1H), 5.85 (br, NH^+), 6.60 (d, 2H), 7.15 (t, 1H). IR (KBr disc): 3441, 2449, 1760, 1608, 1586, 1497, 1483, 1443 cm^{-1}.

Exampl 8. HYPNOTIC ACTIVITY

The hypnotic potency of the α-amino acid phenyl ester derivatives of the invention was determined upon their intravenous administration in mice. The dose required to cause a loss of righting reflex for a minimum period of 30 seconds in 50% of treated mice aft r intravenous injection over 10 seconds was determined. This dose is termed the HD_{50} (hypnotic dose $_{50}$) and is expressed in $\mu mol.kg^{-1}$ These *in vivo* experiments were carri d out as described in detail by Anderson et al., J.Med.Chem. 1997, 40, 1668-1681. The *in vivo* HD_{50} data for a number of compounds of the invention are given in Table I.

The *in vitro* effect of the compounds of the invention at $GABA_A$ receptors was assessed through determination of their ability to inhibit [^{35}S]-TBPS ([^{35}S]-*tert*-butyl bicyclophosphorothionate) binding to rat whole brain membranes. The concentration of α-amino acid phenyl ester derivative required to inhibit 50% of binding of [^{35}S]-TBPS was determined. These *in vitro* experiments were carried out as described in detail by Anderson et al., J.Med.Chem. 1997, 40, 1668-1681. IC_{50} data for a number of compounds of the invention are given in Table I.

TABLE I



Formula I

Example[1]	R_3	R_4	R_5	R_6	TBPS IC_{50} µM	HD50 µmol.kg^{-1}
7a	CH_3	CH_2-CH_3	CH_2-CH_2-O-CH_3	CH_2-CH_2-O-CH_3	22	21
7v	#	#	#	#	14	35
7b	#	#	#	#	18	22
7c	H	CH_2-CH_3	CH_2-CH_2-O-CH_3	CH_2-CH_2-O-CH_3	10	19
7l	H	CH_2-CH_3	CH_2-CH_2-O-CH_2-CH_3	CH_2-CH_2-O-CH_2-CH_3	ND	29
7z	#	#	#	#	18	19
7aa	#	#	#	#	11	22
7s	H	CH_2-CH_2-CH_3	CH_2-CH_2-O-CH_3	CH_2-CH_2-O-CH_3	4.2	12
7d	H	CH_3	CH_2-CH_2-O-CH_2-CH_3	CH_2-CH_2-O-CH_2-CH_3	≤100	68
7x	#	#	#	#	<100	46
7w	#	#	#	#	~100	35
7e	CH_3	CH_3	CH_2-CH_2-O-CH_3	CH_2-CH_2-O-CH_3	<100	52
7f	H	CH_3	CH_2-CH_2-O-CH_3	CH_2-CH_2-O-CH_3	<100	55
7y	CH_3	CH_3	CH_2-CH_2-O-CH_3	CH_2-CH_2-O-CH_3	<100	18
7g	CH_3	CH_3	CH_3	benzyl	22	45
7h	#	#	#	#	27	56
7k	H	CH_2-CH_3	CH_3	benzyl	13	40
7m	H	CH_3	CH_3	CH_2-CH_2-phenyl	59	38
7p	CH_3	CH_3	CH_3	CH_2-CH_2-O-CH_3	~100	72
7i	CH_3	CH_3	CH_3	CH_2-CH=CH_2	~100	60
7j	CH_3	CH_3	CH_2-CH_3	CH_2-CH_3	<50	43
7t	OCH_3	CH_2-CH_3	CH_2-CH_2-O-CH_3	CH_2-CH_2-O-CH_3	14	21
7n[2]	H	CH_2-CH_3	CH_2-CH_2-O-CH_3	CH_2-CH_2-O-CH_3	20	27
7u	H	$CH(CH_3)_2$	CH_2-CH_2-O-CH_3	CH_2-CH_2-O-CH_3	100	27
7o[3]	H	CH_2-CH_3	CH_2-CH_2-O-CH_3	CH_2-CH_2-O-CH_3	<100	64
Reference*					766	139

[1]: R_1 and R_2 are each OCH_3, if not otherwise indicated;

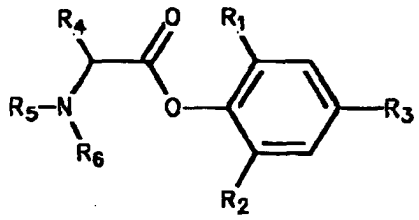
[2]: R_1 and R_2 are each OCH_2-CH_3;

[3]: R_1 and R_2 are each $OCH(CH_3)_2$

*: Reference: 2,6-dimethoxyphenyl 2-morpholinopropionate (GB Patent 1,160,468)

Claims.

1. An α-amino acid phenyl ester derivative having the general formula I



Formula I

wherein

R_1 is (C_{1-3})alkyloxy;

R_2 is (C_{1-3})alkyl, (C_{1-3})alkyloxy or (C_{2-3})alkenyl;

R_3 is hydrogen, (C_{1-3})alkyl, (C_{1-3})alkyloxy or (C_{2-3})alkenyl;

R_4 is (C_{1-6})alkyl;

R_5 and R_6 are independently (C_{1-6})alkyl, (C_{2-6})alkenyl, (C_{2-6})alkynyl or aralkyl, each of which may be optionally substituted with (C_{1-3})alkyloxy, (C_{1-3})alkyloxycarbonyl, cyano or NR_7R_8;

R_7 and R_8 are independently (C_{1-6})alkyl; or a pharmaceutically acceptable salt thereof, with the exclusion of 2,6-dimethoxyphenyl 2-(diethylamino)propionate and 2,6-dimethoxyphenyl 2-(diethylamino)butyrate.

2. The α-amino acid phenyl ester derivative of claim 1, wherein R_1 and R_2 are methoxy; and R_4 is (C_{2-3})alkyl.

3. The α-amino acid phenyl ester derivative of claim 1 or 2, wherein R_3 is hydrogen or methyl; and R_5 and R_6 are methoxyethyl or ethoxyethyl.

4. The α-amino acid phenyl ester derivativ of claim 1, wher in R_1 and R_2 are methoxy; R_3 is hydrogen or methyl; R_4 is ethyl; and R_5 and R_6 are methoxyethyl.

5. Th α-amino acid phenyl ester derivatives of claim 4, wherein the configuration at the α-carbon atom is that of the *R*-enantiomer.

6. An α-amino acid phenyl ester derivative having the general formula I, with the exclusion of 2,6-dimethoxyphenyl 2-(diethylamino)propionate and 2,6-dimethoxyphenyl 2-(diethylamino)butyrate, for use in therapy.

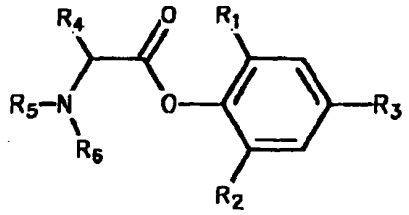
7. A pharmaceutical composition comprising an α-amino acid phenyl ester derivative having the general formula I, or a pharmaceutically acceptable salt thereof, in admixture with pharmaceutically acceptable auxiliaries.

8. The use of an α-amino acid phenyl ester derivative having the general formula I, or a pharmaceutically acceptable salt thereof, for the manufacture of a medicament having hypnotic activity.

9. The use of an α-amino acid phenyl ester derivative having the general formula I, or a pharmaceutically acceptable salt thereof, for the manufacture of a medicament having sedative or analgesic activity, or for the treatment of GABA related diseases, such as anxiety (e.g. panic attack), stress, sleep disorders, post natal depression, and premenstrual tension, and in the alleviation of seizure.

Abstract:

The present invention relates to α-amino acid phenyl ester derivatives having the general formula I



wherein R_1 is (C_{1-3})alkyloxy; R_2 is (C_{1-3})alkyl, (C_{1-3})alkyloxy or (C_{2-3})alkenyl; R_3 is hydrogen, (C_{1-3})alkyl, (C_{1-3})alkyloxy or (C_{2-3})alkenyl; R_4 is (C_{1-6})alkyl; R_5 and R_6 are independently (C_{1-6})alkyl, (C_{2-6})alkenyl, (C_{2-6})alkynyl or aralkyl, each of which may be optionally substituted with (C_{1-3})alkyloxy, (C_{1-3})alkyloxycarbonyl, cyano or NR_7R_8; R_7 and R_8 are independently (C_{1-6})alkyl; or a pharmaceutically acceptable salt thereof. The invention also relates to pharmaceutical compositions comprising said derivatives, and to the use of these α-amino acid phenyl ester derivatives as hypnotics for the induction and maintenance of general anaesthesia.